

A1
cont.

5' to 3' order): (1) WRQTRKD (SEQ ID NO: 1); (2) HYAKNPI (SEQ ID NO: 2); (3) ATINKSL (SEQ ID NO: 3); (4) RRRGMAI (SEQ ID NO: 4); (5) THRLPSR (SEQ ID NO: 5); and (6) TKHGPRK (SEQ ID NO: 6). Several peptides that bind to the NAC portion are: (1) SLKRLPK (SEQ ID NO: 7); (2) RLRGRNQ (SEQ ID NO: 8); (3) WPFHHR (SEQ ID NO: 9); (4) HLYHHKT (SEQ ID NO: 10); (5) THIHHS (SEQ ID NO: 11); and (6) MMMMRL (SEQ ID NO: 12). The NAC portion was chosen because this piece of the protein has been found to aggregate in amyloid plaques in Alzheimer's disease. Particularly preferred, because of stronger binding properties, are THRLPSR (SEQ ID NO: 5); SLKRLPK (SEQ ID NO: 7); THIHHS (SEQ ID NO: 11) and MMMMRL (SEQ ID NO: 12). Most preferred is the peptide SLKRLPK (SEQ ID NO: 7).

Please delete the paragraph on page 16, lines 23-27, thru page 17, lines 1-9, and replace it with the following paragraph:

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α -Synuclein (wildtype, A53T and A30P) was cloned into the NotI site of pcDNA3. The sequence of each construct was confirmed by DNA sequencing. For production of recombinant protein, α -synuclein was inserted into the NcoI/NotI site of the Pro-Ex His 6 (SEQ ID NO: 13) vector (GIBCO/BRL). To generate recombinant α -synuclein, Bper (Pierce) reagent was used to solubilize the recombinant α -synuclein from the IPTG-induced bacterial lysates, which were then passed over a nickel-agarose affinity column, washed and eluted with imidazole according to the manufacturer's directions (GIBCO/BRL). Following purification, the His-6 tag (SEQ ID NO: 13) was cleaved with TEV protease and removed by passing through a nickel-agarose column. Antibodies used include: polyclonal anti α -synuclein (SC1, 1:2000 for immunoblotting and 1:500 for immunocytochemistry against human α -synuclein, residues 116-131, sequence = MPVDPDNEAYEMPSEE) (SEQ ID NO: 14), monoclonal anti α -synuclein-1 (1:1000, Transduction Labs), polyclonal rabbit anti-ubiquitin (1:1000 for immunoblotting and 1:500 for immunocytochemistry, Dako).

Please delete the paragraph on page 31, lines 25-28, thru page 32, lines 1-2, and replace it with the following paragraph:

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Phage display (phage display kit was from New England Biolabs (Beverly, MA)) was used to specifically select for peptides that can inhibit iron binding. Phages were identified from libraries that bind to amino acids 121-131 and amino acids 61 - 87 of α -synuclein and partially inhibit iron-induced aggregation of α -synuclein. The peptides were selected using the α -synuclein 121-131 and 61-87 peptides as the bait. One such peptide has the sequence SLKRLPK (SEQ ID NO: 7).

{ Please delete the paragraph on page 32, lines 3-12, and replace it with the following paragraph: }

To demonstrate that the peptide SLKRLPK (SEQ ID NO: 7) binds α -synuclein, the α -synuclein was absorbed to a plastic well, phage was added at dilutions of 1: 10, 1: 100, and 1: 1000, incubated 1 hour and washed 5 times. Bound phage was detected by adding peroxidase coupled anti-phage antibody, incubating 1 hr, washing 5 times and detecting signal using the peroxidase substrate ABTS. Bound phage produces a dark green signal that is measured as optical density with a spectrophotometer. Using these conditions, the phage containing the SLKRLPK (SEQ ID NO: 7) peptide gave OD's of 0.882, 0.844, and 0.480 at dilutions of 1: 10, 1: 100, and 1: 1000, respectively. By contrast, another phage that did not bind gave OD's of 0.019, -0.027, and -0.554 respectively.

Please delete the paragraph on page 32, lines 18-25, and replace it with the following paragraph:

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The peptide has the sequence "SLKRLPK" (SEQ ID NO: 7), and corresponds to a sequence expressed by a phage that shows particularly strong binding to α -synuclein by ELISA. The sequence